CLAIMS

1. A method of identifying an agent which modulates 2-oxoglutarate dependent oxygenase activity, the method comprising:

- contacting a 2-oxoglutarate dependent oxygenase and a test agent in the presence of a substrate comprising one or more ankyrin repeat, or fragment thereof, in conditions under which the substrate is hydroxylated in the absence of the test agent; and

- determining hydroxylation of the substrate

thereby determining whether or not the agent modulates 2-oxoglutarate dependent oxygenase activity.

2. A method according to claim 1, wherein the substrate is hydroxylated at an asparagine residue.

3. A method according to claim 2, wherein the asparagine residue is part of a valine-asparagine, aspartate-valine-asparagine, isoleucine-asparagine or leucine-asparagine sequence.

4. A method according to any one of the preceding claims, wherein the substrate is IκB-α, p105, FEM-1, p19-INK-4d, GABPbeta, Tankyrase 1/2, 2-5A-d-R, Gankyrin, Myotrophin, M110, FGIF (Factor Inducing Foetal Globin), or a fragment of any thereof.

5. A method according to claim 4, wherein the substrate is p105 or a fragment thereof comprising Asn 778 of p105 or a peptide analogue of p105 or fragment thereof comprising an asparagine equivalent to Asn 778 of p105 and wherein hydroxylation of Asn 778 or of a said equivalent asparagine is determined.

6. A method according to any one of the preceding claims, wherein the 2-oxoglutarate dependent oxygenase is a JmjC protein.

7. A method according to claim 6, wherein the JmjC protein is factor inhibiting hypoxia-inducible factor (FIH).

8. A method according to any one of the preceding claims, wherein the hydroxylation of the substrate is determined by monitoring 2-oxoglutarate turnover.

9. A method according to any one of claims 1 to 7, wherein the hydroxylation of the substrate is determined by mass spectrometry.

10. A method according to any one of claims 1 to 7, wherein the hydroxylation of the substrate is determined by monitoring for transcription or expression of a reporter gene driven by a promoter regulated by an ankyrin repeat protein.

11. A method according to any one of the preceding claims further comprising formulating an agent identified as a modulator of 2-oxoglutarate dependent oxygenase activity with a pharmaceutically acceptable recipient.

12. A method of identifying an agent which selectively modulates activity of a first 2-oxoglutarate dependent oxygenase, the method comprising:

(a)(i) contacting a first 2-oxoglutarate dependent oxygenase and a test agent in the presence of a substrate comprising one or more ankyrin repeat, or fragment thereof, in conditions under which the substrate is hydroxylated in the absence of the test agent; and

(ii) determining hydroxylation of the substrate;

(b)(i) contacting a second 2-oxoglutarate dependent oxygenase and a test agent in the presence of a substrate comprising one or more ankyrin repeat, or fragment thereof, in conditions under which the substrate is hydroxylated in the absence of the test agent; and

(ii) determining hydroxylation of the substrate;

thereby determining whether or not the agent modulates activity of the first 2-oxoglutarate dependent oxygenase.

13. A method according to claim 12, wherein the test agent inhibits activity of the first 2-oxoglutarate dependent oxygenase.

14. A method according to claim 12 or 13, wherein the first 2-oxoglutarate dependent oxygenase is FIH.

15. A method according to any one of claims 12 to 14, wherein the second 2-oxoglutarate dependent oxygenase is a PHD.

16. A method according to claim 12 or 13, wherein the first 2-oxoglutarate dependent oxygenase is a PHD.

17. A method according to any one of claims 12, 13 and 16, wherein the second 2-oxoglutarate dependent oxygenase is FIH.

18. A method according to any one of claims 12 to 17, wherein the substrate is as defined in any one of claims 2 to 5.

19. A method of identifying an agent which selectively modulates 2-oxoglutarate dependent oxygenase activity on a first substrate, the method comprising:

(a)(i) contacting a 2-oxoglutarate dependent oxygenase and a test agent in the presence of a first substrate, or fragment thereof, in conditions under which the substrate is hydroxylated in the absence of the test agent; and

(ii) determining hydroxylation of the first substrate; and

(b)(i) contacting a 2-oxoglutarate dependent oxygenase and a test agent in the presence of a second substrate, or fragment thereof, in conditions under which the substrate is hydroxylated in the absence of the test agent; and

(ii) determining hydroxylation of the second substrate;

wherein at least one of said first and second substrates comprises one or more ankyrin repeat;

thereby determining whether or not the agent selectively modulates 2-oxoglutarate dependent oxygenase activity on a first substrate.

20. A method according to claim 19, wherein the first and/or second substrate comprising one or more ankyrin repeat is as defined in any one of claims 2 to 5.

21. A method according to claim 19 or 20, wherein the first substrate is HIF and the second substrate comprises one or more ankyrin repeat.

22. A method according to claim 19 or 20, wherein the second substrate is HIF and the first substrate comprises one or more ankyrin repeat.

23. A method according to claim 19 or 20, wherein the first and second substrates are different and each comprises one or more ankyrin repeat.

24. A method according to any one of claims 19 to 23, wherein the 2-oxoglutarate oxygenase is as defined in claim 6 or 7.

25. A method according to any one of claims 1 to 24, wherein the test agent is a polypeptide comprising an ankyrin repeat or an analogue thereof.

26. A method according to claim 25, wherein the analogue is an ankyrin repeat that lacks an asparagine residue capable of being hydroxylated by 2-oxoglutarate dependent oxygenase.

27. An agent identified by an assay method according to any one of the preceding claims.

28. An agent according to claim 27 for use in a method of treatment of the human or animal body by therapy.

29. Use of an agent according to claim 27 in the manufacture of a medicament for the treatment of a condition associated with increased or decreased levels or activity of an ankyrin repeat-containing protein or the treatment of a condition where it is desired to modulate activity of an ankyrin repeat-containing protein.

30. Use according to claim 29, wherein the ankyrin repeat-containing protein is IκB-α, p105, FEM-1, p19-INK-4d, GABPbeta, Tankyrase 1/2, 2-5A-d-R, Gankyrin, Myotrophin, M110 or FGIF.

31. Use according to claim 29 or 30, wherein said condition is selected from the group consisting of ischemia, cancer, inflammatory disorders, immune disorders, anaemia and beta thalassemia.

32. A method of treating a condition associated with increased or decreased levels or activity of an ankyrin repeat-containing protein or the treatment of a condition where it is desired to modulate activity of an ankyrin repeat-containing protein comprising administering a therapeutically effective amount of an agent according to claim 27 to an individual in need thereof.

33. A method of modulating ankyrin repeat-containing protein mediated activity in a cell comprising contacting the cell with a substance which inhibits the asparagine hydroxylase activity of a 2-oxoglutarate dependent oxygenase.

34. A polpeptide comprising a ankyrin repeat sequence analogue which is not susceptible to hydroxylation by a 2-oxoglutarate oxygenase for use in a method of treatment of the human or animal body.

35. The use of a polypeptide comprising an ankyrin repeat or analogue thereof, or a polynucleotide encoding said polypeptide, in an *in vitro* or *in vivo* method of inhibiting a 2-oxoglutarate oxygenase.

36. A method according to claim 35, wherein the 2-oxoglutarate oxygenase is as defined in claim 6 or 7.

37. A method according to claim 35 or 36, wherein the polypeptide is IκB-α, p105, FEM-1, p19-INK-4d, GABPbeta, Tankyrase 1/2, 2-5A-d-R, Gankyrin, Myotrophin, M110, FGIF (Factor Inducing Foetal Globin), or a fragment of any thereof.